sonography. Fat-suppressed 3D spoiled gradient-echo MRI was also used to get the reference value. The joint space width (JSW) and Kellgren and Lawrence (K-L) grade were measured in weight-bearing anteroposterior knee radiograph. The Kappa and intraclass correlation coefficient (ICC) were used to determine inter- and intra-observer agreement of the US measurements.

**Results:** In medial femoral condyle, the opportunity to obtain cartilage thickness was increased significantly using the longitudinal US scan as compared with tansverse scan (48 cases vs. 36 cases, $p < 0.05$). There was a good correlation between longitudinal US scan and MRI in the maximum and minimum cartilage thicknesses of medial condyle ($r = 0.568$; $r = 0.844$, respectively, $p < 0.01$). However, there was no correlation between suprapatellar transverse US scan and MRI in medial condyle. In lateral condyle, both US scans showed good correlations with MRI. In Bland-Altman analysis, longitudinal US scan showed good agreement with MRI except in the minimal cartilage thickness of lateral condyle. There was high overall intra- and inter-observer agreement in US scan.

**Conclusions:** US scan in the longitudinal plane is a more feasible method than suprapatellar transverse scan for measuring cartilages thickness of medial femoral condyle in knee OA patient.

## 381 SECOND HARMONIC GENERATION IMAGING AND COLLAGENEOUS MATRIX MODIFICATION IN OSTEOARTHRITIS DISEASE

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**Purpose:** In healthy cartilage, collagen fibers are pseudo-randomly distributed and interact with a gel of Proteoglycans. Degenerative disease like osteoarthritis can affect the organization of the extracellular matrix (ECM) surrounding chondrocytes leading to a modification or even degradation of the collagen network. The aim of this work was to characterize the remodelling of the collagen network under mechanical or biochemical stress.

**Methods:** Near infrared tomography (Multiphoton Excitation, Second Harmonic Generation SHG, Fluorescence Lifetime imaging Microscopy FLIM) represents an appropriate tool for cartilage imaging due to its advantages in terms of depth penetration. In the ECM collagen fibers give rise to a strong SHG signal (high non linear susceptibility) and Proteoglycans show a high level of autofluorescent after multiphoton excitation.

Under mechanical stress, a remodeling of the collagen network occurs and can be comparable to disturbance occurring in disease. To characterize structural modification on the arrangement of collagen fibers in the ECM, we used image analysis based on co-occurrence matrix (Haralick). Textural parameters can give information like homogeneity ('Angular Second Moment') or size of textural elements ('Inverse Difference Moment', 'Correlation'). We followed their evolution when samples were submitted to mechanical (compression) or biochemical (Collagenase) stress.

**Results:** It came out that the behavior of the collagen network was different under compression or enzymatic action.

Enzymatic action of Collagenase lead to a loss of SHG signal according to time of incubation: this evolution can either be attributed to a loss of collagen content or to a modification of collagen molecules affecting their non linear susceptibility. By this way, we proved that the SHG signal came specifically from collagen in cartilage samples.

Samples submitted to compression were characterized by higher 'Correlation', associated with a decrease of 'IDM' and 'ASM'. Those evolutions suggest the presence of long linear structures, an effect of packing of collagen fibrils and the apparition of nodes where the density of collagen is important versus areas showing a lack of molecules. Moreover the ECM seemed more dense and compact and SHG signal was even more intense.

We also were interested in the pericellular matrix of chondrocytes containing type VI Collagen. This molecule acts as a transducer of biochemical or biomechanical signals and hypothesis have been emitted about its protective role. Moreover, during osteoarthritis, its content in the pericellular area increases when compared to healthy specimens. Thus Collagen VI can be considered as a biomarker characterising disease states. FLIM associated to Spectral and SHG analysis confirmed the presence of Collagen VI in the pericellular matrix of chondrocytes.

**Conclusions:** SHG, FLIM and Spectral Imaging combined with multiphoton excitation enable tissue imaging at deep penetration. The association of all this imaging modalities represents a potential diagnostic tool for cartilage disease, since it enables to detect local modification of the collagen network of the ECM without any labelling (SHG) and the presence of collagen VI in the lacunae around cells. Moreover these imaging techniques can be used to validate the well functionality of bioconstructs by following synthesis of collagen for instance.

This work was partly supported by a grant of CG54 and Region de Lorraine.

## 382 COMPARISON OF ONE YEAR CHANGE IN MINIMUM JOINT SPACE WIDTH TO FIXED LOCATION JOINT SPACE MEASUREMENTS IN LYON SCHUSS X-RAYS FROM THE A9001140 STUDY

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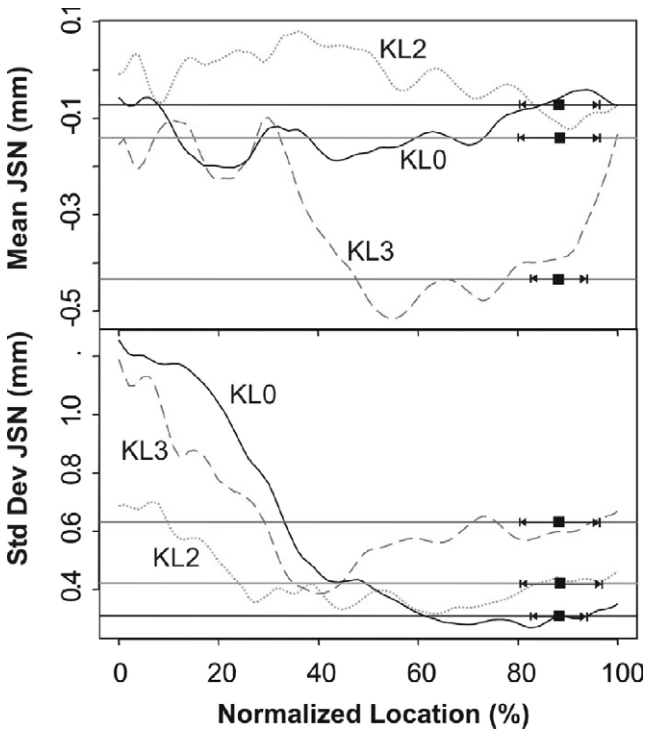
**Purpose:** Joint space narrowing (JSN) calculated from the change in minimum joint space width (mJSW) from x-ray has become a valuable tool for the monitoring of progression in osteoarthritis (OA). The goal of this analysis was to investigate the relative sensitivity of JSN when measured at fixed locations in Lyon schuss x-rays.

**Methods:** Lyon schuss x-rays from a subset of 67 subjects from the A9001140 study acquired at 7 sites at baseline and 12 months were analyzed using KneeAnalyzer (Optasia Medical), proprietary statistical model-based analysis software. The femoral and tibial margins of the medial compartment were segmented and JSW was measured along a normalized distance across the medial compartment from 0% at the tibial spine to 100% at the medial margin of the tibia. 31 subjects had Non-OA defined as Kellgren and Lawrence (KL) grades of 0 or 1. The OA subjects had KL = 2 (n = 17) or KL = 3 (n = 19). The JSN at the mJSW location and the average JSN between 51%-90% (aJSN 51−90%) were calculated along with the standard deviation (Std Dev) and the standard response mean (SRM). Significance was determined by $p < 0.05$.




**Results:** Figure 1 shows the mean (top) and standard deviation (bottom) of the JSN for each KL group across the medial compartment. The squares are the location of the mJSW measurement (± one Std Dev). Between 50 and about 85% the profiles are relatively flat indicating a consistent difference in JSN. However, around 90% (the approximate

location of mJSW) there is a steep change in the JSN profile for KL3 subjects and a crossing of the Non OA and KL2 plots. The Std Dev of the measurements, are relatively consistent from 50 to 100%.

| | mJSW narrowing | | | | aJSN 51−90% | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Std Dev | SRM | p | Mean | Std Dev | SRM | p |
| KL0 | −0.07 | 0.31 | −0.235 | 0.20 | −0.12 | 0.25 | −0.478 | 0.01 |
| KL2 | −0.14 | 0.42 | −0.336 | 0.19 | −0.04 | 0.34 | −0.124 | 0.62 |
| KL3 | −0.43 | 0.63 | −0.687 | 0.008 | −0.45 | 0.56 | −0.795 | 0.003 |

**Conclusions:** This study is unique in that it measured the JSN profile from Lyon schuss X-rays. The range of 51%-90% for the aJSN measurements was chosen because it was an area of fairly consistent narrowing as well as low Std Dev for the measurements. For KL3, both mJSN and aJSN 51−90% showed significant change but the latter had a higher SRM and hence is potentially more sensitive. While the Non-OA had a significant and unexpected change in aJSN 51−90% it should be noted that direct comparisons between the two methods were not significantly different. The aJSN measures may provide additional information regarding OA progression in the medial compartment to mJSW narrowing alone and further investigation is warranted to determine how these measures reflect the complicated underlying biological progression of OA.

## 383 ASSESSMENT OF CARTILAGE UNLOADING BY QUANTITATIVE T2 MAPPING OF KNEE CARTILAGE: INITIAL RESULTS ON THE DIFFERENTIATION OF HEALTHY AND ALTERED ARTICULAR CARTILAGE

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**Purpose:** Biomechanical behaviour of articular cartilage in-vivo is hardly to assess. However, taking into consideration that walking and standing is loading, hence deformation of collagen structure and water content changes after rest. T2 mapping is an adequate approach for the evaluation of collagen structure and water content. The goal was to use T2 mapping and its zonal assessment for evaluation of unloading during a one hour MR scan in healthy volunteers, patients with cartilage defect and in the follow-up after cartilage repair (MACT).
**Methods:** 3T MRI was performed using a multi-echo spin-echo (SE) in 10 volunteers and Twenty-five patients: Eight patients pre-operatively with a single partial thickness cartilage defect on the femoral condyle and 17 patients 20.7±16.9 months after MACT. T2 relaxation times were obtained using a pixel wise, mono-exponential (NNLS) fit analysis. T2 sequence was obtained at the beginning (pre-scan) and at the end (post-scan) of the clinical MR examination, including high resolution morphological scan. The time gap between both T2 measurements was 45 minutes. The patients were asked to not rest before the MR scan, so daily activity and walking to the scanner was used as loading. were enrolled in this study. within the knee. Regions of interest (ROI) analysis were manually done in consensus and areas of cartilage damage/cartilage repair and healthy control cartilage were identified using the morphological images as well as surgical reports. All areas were located within the weight bearing zone of the femoral cartilage. For further evaluation on the zonal variation, the ROIs were divided into two equal sized deep and superficial regions.
**Results:** For healthy seen cartilage quantitative T2 values for the pre-unloading evaluation were 50.9±13.1 ms for the deep zone and 56.0±10.9 ms for the superficial zone. After 45 minutes T2 values showed not significant increase to 51.2±13.3 ms for the deep aspects and 57.7±12.8 ms for the superficial aspect. The increase between deep and superficial T2 values was significant. Pre-operative patients show initial significant lower T2 values with 41.5±6.0 ms for the deep zone and 50.4±6.4 ms for the superficial zone, however after unloading, values rise significantly to 52.6±14.3 for the deep and 58.5±14.3 for the superficial aspect. The cartilage repair tissue after MACT showed pre values of 51.9±11.6 ms for the deep cartilage zone and 55.9±14.1 for the superficial (Pre and Post T2 and Substraction Image shown in Figure 1). Thus there was no significant difference from the healthy seen cartilage sites, the post-unloading scan showed a significant increase in T2 values for deep (56.3±14.1 ms) and even clearer for superficial (60.8±18.5 ms) aspects of cartilage repair tissue.
**Conclusions:** Quantitative T2 relaxation can be used to assess pre- and post unloading values of articular cartilage in a clinical setup. Furthermore the presented approach of two SE-T2 scans at the beginning and the end of an MR scan might give additional information on the constitution of cartilage and might help to differentiate between healthy and affected articular cartilage. However it appears that changes in water content and anisotropy over time are different in healthy cartilage compared to altered cartilage. Larger patient groups have to elucidate a potential clinical impact of the presented approach.

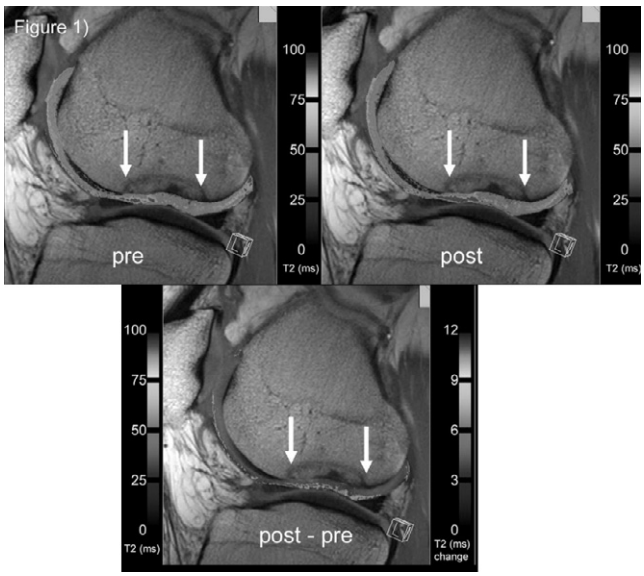


Figure 1. Quantitative T2 Map of a patient after MACT: Top left image shows the pre-unloading scan, top right image the post-unloading scan. The image at bottom shows the substracted T2 map (post − pre value), with changed repair tissue.

## 384 MAGNETIC RESONANCE IMAGING FOR THE MEASUREMENT OF STRUCTURE MODIFICATION IN KNEE OSTEOARTHRITIS

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**Purpose:** The objective of this pilot study was to explore the structure modifying effect of Structum® (Chondroitin Sulfate) in knee osteoarthritis (KOA) using quantitative and qualitative magnetic resonance imaging (MRI).
**Methods:** Multicenter, double-blind, placebo-controlled, parallel-group study. Forty-three patients over 50 years with KOA (ACR criteria), graded II or III on Kellgren-Lawrence scale, were randomized to receive either Structum® 500 mg (N = 22) or placebo (N = 21) twice daily for 48 weeks. Clinical symptomatology was assessed throughout the Lequesne index and VAS for pain during daily activities, at baseline 24 and 48 weeks. In parallel 3D MRI imaging was acquired at baseline, 24 and 48 weeks. Global and compartments cartilage volume was quantified as well as other MRI features such as joint cartilage abnormalities, meniscal lesions, ligaments abnormalities, synovitis, synovial effusion, osteophytes, subchondral cysts, popliteal cysts and subchondral oedema. Intra- and inter-reproducibility of MRI was tested by the Spearman correlation coefficient for quantitative assessments and by the kappa coefficient for qualitative assessments. Treatment groups were compared by an analysis of covariance (ANCOVA) with baseline value as a covariate.
**Results:** Demographic data for the Structum® and placebo groups were as follow: mean (SD) age 63.6 (8.2) and 66.5 (8.1) respectively, female sex 72.7% and 57.1%. At baseline clinical signs and total volume of cartilage were comparable in both groups: Mean Lequesne index was 9.6 (3.4) in the Structum® group and 10.4 (3.6) in the placebo, pain VAS 50.9 (15.9) and 55.4 (14.5) and mean total cartilage volume was 12969.8 $mm^3$ (3657.7) and 13916.9 $mm^3$ (4439.2). At baseline we observed a highly significant correlation for the assessment of cartilage volumes, number of cysts and osteophytes, the Spearman coefficients ranging from 0.951 to 0.980 for the within investigator and from 0.714 to 0.957 for the between investigator. Between investigator reproducibility